Amendments to the Specification

It is noted that the subject patent application has been published as United States Patent Application Publication, Pub. No.: US 2006/0108229 A1, published May 25, 2006.

Amendments to the specification set forth herein are made with reference to said Pub. No.: US 2006/0108229 A1, published May 25, 2006, hereinafter referred to as the Published Application.

Herein, with amendments to the specification, please note that "strikeout" matter is shown with larger-than-normal italic letters containing the strikeout horizontal marks such as in this example: *strikeout*.

Please amend the previously filed Abstract of the Published Application as follows:

An object of the invention is to provide an electroporation method for treating vesicles with exogenous material for insertion of the exogenous material into the vesicles which includes the steps of: a. retaining a suspension of the vesicles and the exogenous material in a treatment volume in a chamber which includes electrodes, wherein the chamber has a geometric factor (cm.sup.-1) defined by the quotient of the electrode gap squared (cm.sup.2) divided by the chamber volume (cm.sup.3),

wherein the geometric factor is less than or equal to 0.1 cm.sup.-1, wherein the suspension of the vesicles and the exogenous material is in a medium which is adjusted such that the medium has conductivity in a range spanning 0.001 to 100 ~~0.001 to 100 milliSiemens~~, wherein the suspension is enclosed in the chamber during treatment, and h. treating the suspension enclosed in the chamber with one or more pulsed electric fields. With the method, the treatment volume of the suspension is scalable, and the time of treatment of the vesicles in the chamber is substantially uniform.

Please amend the previously filed enumerated paragraph [0009] of the Published Application as follows:

[0009] Typical cell densities used are in the range of 1 million to 10 million cells per milliliter. The cells are typically placed in a physiological medium with high ionic content such as phosphate buffered saline, which has a conductivity of 0.017 Siemens/cm ~~(17,000 mS/cm) per centimeter~~ (17,000 microSiemens/cm).

Please amend the previously filed enumerated paragraph [0073] of the Published Application as follows:

[0073] To achieve the foregoing and other advantages, the present invention, briefly described, provides a static chamber

with large volume to insure all cell are subject to the same electric field intensity and direction and the density of the cells and material are uniform. With this invention any waveform may be used. This invention is a voltage waveform generator connected to an electrode with parallel plates with has low conductivity media, a cell density of 20 million cells per 10 milliliters or less. The invention uses media with conductivity between 10 microSiemens/cm and 100 milliSiemens/cm as shown in FIG. 2 ~~50 - mu S/cm and 500 - mu S/cm~~. The invention may be used in clinical applications and has a closed sterile chamber into which the cells and large molecules are inserted and removed.

Please amend the previously filed enumerated paragraph [0075] of the Published Application as follows:

[0075] a. retaining a suspension of the vesicles and the exogenous material in a treatment volume in a chamber which includes electrodes, wherein the chamber has a geometric factor (cm.sup.-1) defined by the quotient of the electrode gap squared (cm.sup.2) divided by the chamber volume (cm.sup.3), wherein the geometric factor is less than or equal to 0.1 cm.sup.-1), wherein the suspension of the vesicles and the exogenous material is in a medium which is adjusted such that the medium has conductivity in

a range spanning 0.001 to 100 milliSiemens as shown in FIG.2, wherein the suspension is enclosed in the chamber during treatment, and

Please amend the previously filed enumerated paragraph [0080] of the Published Application as follows:

[0080] The vesicles can be living cells, and the medium can be a physiological medium and has a conductivity between 10 micro and 1000 microS/cm as shown in FIG. 2. The number of living cells that are treated in the chamber at one time can be more than 10 million in number. Furthermore, the number of living cells that are treated in the chamber at one time can be more than 20 million in number.

Please amend the previously filed enumerated paragraph [0089] of the Published Application as follows:

[0089] In accordance with another aspect of the invention, an electroporation apparatus is provided which includes a chamber which has a chamber volume of at least 2 milliliters. A pair of electroporation electrodes are contained within the chamber. An electroporation medium, carrying vesicles in suspension, is contained in the chamber between the electroporation electrodes. The medium has a conductivity between 10 micro and 1000 microS/cm as shown in FIG. 2. A source of

pulsed voltages is electrically connected to the electroporation electrodes, and means for adding material to the chamber for electroporation treatment therein. Also, means are provided for removing treated material from the chamber.

Please amend the previously filed enumerated paragraph [0108] of the Published Application as follows:

[0108] This present invention specifies a range of material conductivities, which can be used versus the chamber dimensions, the larger the volume the smaller the conductivity. This invention specifies an operating area for use with the larger volume electrodes. This is illustrated in FIG. 2. Operating points of prior art published results are also presented in FIG. 2 as squares. For chambers with a Geometric Factor less than 0.1 there are two limiting factors, which are related. The first is the absolute value of the chamber resistance. In this invention the chamber resistance is one ohm or greater. Operating below one ohm is viewed ~~view~~ as impractical. The other constraint is the conductivity of the medium in the chamber. As the conductivity decreases the charging time of the cell membrane increases because there are fewer ions external to the cell membrane. More specifically with respect to FIG. 2, an "Operating Region of the Invention" is clearly shown to be a triangular region. The topmost point of the triangular

"Operating Region of the Invention" has the coordinates of (along the horizontal axis) Geometric Factor in cm^{-1} of 0.100000 and (along the vertical axis) Conductivity in microSiemens/cm of 100,000.00. In addition, the far right bottommost point of the triangular "Operating Region of the Invention" has the coordinates of (along the horizontal axis) Geometric Factor in cm^{-1} of 0.100000 and (along the vertical axis) Conductivity in microSiemens/cm of 1.00. In addition, for the triangular "Operating Region of the Invention", the resistance R equals 1 ohm. It is noted that 100,000 microSiemens/cm equals 100 milliSiemens/cm. It is also noted that 1.00 microSiemens/cm equals 0.001 milliSiemens/cm.

Please amend the previously filed enumerated paragraph [0122] of the Published Application as follows:

[0122] A component of the invention is the use of low conductivity medium within a defined range to limit amperage and heat while simultaneously providing enough ions to effectively electroporate cells. Typically the medium used will have a conductivity between 10 micro and 1000 microS/cm ~~50-mS/cm and 500-mS/cm~~.

Please amend the previously filed enumerated paragraph [0125] of the Published Application as follows:

[0125] The conductivity of the medium used in electroporation is an important aspect of this invention. In this process, a low conductivity medium is employed to keep the total resistance of the medium small and virtually eliminate heating. There is a limit to the lower conductivity medium that can be used. As the ionic content of the medium is reduced the number of free ions that are available to build charge (voltage) across the cell membrane is decreased. The effect is to increase the amount of time it takes to charge the membrane. This process is described by the equation in Neumann, p71. Assuming a typical cell diameter of 10 microns, the charging time is 20 microseconds at 80 microS/cm 80 mS/cm. Below 80 microS/cm 80 mS/cm the charging time becomes too long and the pathways in cell membranes stop forming.